

What is being claimed is:

1. A method of expressing PCA interacting partners in plant material comprising:

(A) transforming said material with:

(1) a first construct coding for a first fusion product comprising

5 (a) a first fragment of a first molecule whose fragments can exhibit a detectable activity when associated and

(b) a first protein-protein interacting domain; and

(2) a second construct coding for a second fusion product comprising

(a) a second fragment of said first molecule and

10 (b) a second protein-protein interacting domain that can bind (1)(b);

(B) culturing said material under conditions allowing expression of said PCA interacting partners, and

(C) detecting said activity.

15 2. The method of claim 1 wherein the plant material is selected from the group consisting of whole plants and plant-derived organs, tissues, cells, subcellular parts, and protoplasts.

20 3. The method of claim 1 or claim 2 wherein the plant material is derived from a transgenic plant.

4. The method of claim 1 where an inducer is added to facilitate the interaction of said protein-protein interaction domains.

5. The method of claim 1 or 4 wherein a fluorescent substrate is added and said activity is detected using fluorescence microscopy, spectrofluorometry, FACS analysis, or a fluorescence-detecting video system.

5 6. The method of claim 1 or 4 where said plant material is cultured on a selective medium.

7. A system for use as a standard or control in a PCA assay or for use in validating a PCA assay comprising:

10 (a) a first fusion product comprising a fragment of a first molecule whose fragments can exhibit a detectable activity when associated and a first protein-protein interacting domain; and
(b) a second fusion product comprising a second fragment of said first molecule and a second protein-protein interaction domain that interacts with said first protein-protein interaction domain.

15 8. A system according to claim 7 where said first and second protein-protein interaction domains are selected from the group consisting of:

- 1) NPR1 + TGA2,
- 2) FKBP + FRB,
- 3) leucine zippers.

20

9. A PCA assay using any of the systems of claims 7 or 8.

10. A plant-based PCA assay using any of the systems of claims 7 or 8.

11

12 A plant transgenic for one or more genes, each independently selected from the group consisting of:

(A) 1 or more genes coding for 1 or more interacting partners able to participate in a PCA

assay, and

5 (B) 1 or more genes which result, either directly or indirectly, in the presence of 1 or more interacting partners able to participate in a PCA assay.

12 13. A plant according to claim 12 where the plant is of the genus *Arabidopsis*.

10 13

14. A plant according to claim 13 where the plant is *Arabidopsis thaliana*.

14

15. A plant according to claim 12 where the interacting partners comprise one or more of a leucine zipper/reporter molecule fusion, a NPR1/reporter molecule fusion, a TGA2/reporter molecule fusion, a FKBP/reporter molecule fusion or, a FRB/reporter molecule fusion.

15

16. A method of determining whether a mutated gene acts upstream in a pathway affecting an inducible interaction comprising performing a PCA assay in a mutated plant and correlating a change in PCA activity, relative to that measured in a non-mutated control plant, with the presence of one or more genes acting upstream in said pathway.

16

17. A method of identifying one or more genes involved in a pathway controlling an inducible interaction which results in a monitorable activity comprising:

(1) mutagenizing a seed from a transgenic plant expresssing an interacting partner involved in PCA;

(2) germinating the seed;

(3) treating with an inducer that controls the interaction of any interacting partners present, and

(4) monitoring said activity, and

5 (5) correlating said activity with 1 or more genes involved in a pathway controlling an inducible interaction .

17
18. A method of cloning a gene comprising:

10 1) identifying a gene according to the method of claim 17, and

2) cloning said gene.

18
19. A gene cloned by the method of claim 18 or a gene substantially similar thereto.

19
20. A product derived from any of the genes of claim 19.

20
21. A vector comprising the gene of claim 19 or its product.

21
22. Biological material genetically transformed with the gene of claim 19 or the vector of claim 21.

22
23. A method comprising mutating a plant or plant material that exhibits a first level of interaction between PCA interacting partners and selecting for a resultant plant or plant material that exhibits a lower level of said interaction.

24. A plant or plant material formed according to the method of claim 23.

25. A method of identifying plant molecule that functions as a PCA interacting partner in a PCA assay comprising

5 (1) reacting

(A) a library of plant molecules which are fused to a first fragment of a reporter molecule, said first fragment exhibiting low or no activity, with

(B) a bait molecule fused to a second fragment of said reporter molecule, said second fragment also exhibiting low or no activity and

10 (2) correlating reconstitution of reporter molecule activity with the presence of a PCA interacting partner.

26. A method employing a Protein Complementation assay/Universal Reporter System (PCA/URS) for detecting and screening for ligands and/or bioregulators of a plant cellular receptor, which method comprises:

a) generating a first nucleic acid vector encoding a first fusion product comprising:

i) a first fragment of a first PCA/URS reporter molecule, and

ii) a second molecule, fused to said first fragment, which comprises a first subdomain of a cellular receptor molecule of interest;

20 b) generating a second nucleic acid vector encoding a second fusion product comprising:

i) a second fragment of said first PCA/URS reporter molecule, and

ii) a third molecule, fused to said second fragment, which comprises a second subdomain of said cellular receptor, and where said second subdomain may be the same as said first subdomain in the case of a homodimeric cellular receptor, or different from said first

subdomain in the case of a heterodimeric cellular receptor; or a receptor coactivator or a protein;

- c) transfecting eukaryotic cells with said first and second nucleic acid vectors; and
- d) testing said transfected cells for activity of said PCA/URS reporter molecule, said activity indicating reassociation of the first and second fragments of the PCA/URS reporter molecule mediated by the interaction of said first and second subdomains of the cellular receptor molecule; said association being induced by binding said receptor to said ligand or bioregulator.

28

27. A method employing a Protein Complementation Assay/Universal Reporter System (PCA/URS) for detecting and screening for ligands and/or bioregulators of a plant cellular receptor, which method comprises:

- a) generating a first nucleic acid vector encoding a first fusion product comprising:
 - i) a first fragment of a first PCA/URS reporter molecule, and
 - ii) a second molecule, fused to said first fragment, which comprises a first subdomain of a cellular receptor molecule of interest;
- b) generating a second nucleic acid vector encoding a second fusion product comprising:
 - i) a second fragment of said first PCA/URS reporter molecule, and
 - ii) a third molecule, fused to said second fragment, which comprises a second subdomain of said cellular receptor, and where said second subdomain may be the same as said first subdomain in the case of a homodimeric cellular receptor, or different from said first subdomain in the case of a heterodimeric cellular receptor;
- c) transfecting eukaryotic cells with said first and second nucleic acid vectors;
- d) obtaining a clonal population of cells that express said first and second fusion products; and
- e) testing said transfected cells for activity of said PCA/URS reporter molecule, said

activity indicating reassociation of the first and second fragments of the PCA/URS reporter molecule mediated by the interaction of said first and second subdomains of the cellular receptor molecule; said association being induced by binding said receptor to said ligand or bioregulator.

add a3

09251024 020041
TOP SECRET - FEDERAL BUREAU OF INVESTIGATION